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Comparative Development of *Heliothis virescens* and Hybrid Backcross Progeny Reared at Five Temperatures

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Comparative Development of *Heliothis virescens* and Hybrid Backcross Progeny Reared at Five Temperatures

By J. W. Smith,[1] E. G. King,[1] and D. W. Parvin[2]

ABSTRACT

Heliothis virescens (F.) and backcross progeny from the intercross of *H. virescens* males and *H. subflexa* (Guenée) females were reared on a 14- to 10-h light-to-darkness schedule in environmental cabinets held at 16°, 20°, 25°, 29.5°, and 34° C, with relative humidity at 60% for eggs, larvae, and pupae and at 80% to 85% for adults. Eggs (12-h-old) used in viability and incubation tests were placed individually in 30-ml cups containing about 5 ml of 3% agar. Each temperature treatment was replicated 5 times with 21 cups per replication. The cups were checked for newly hatched larvae at 8-h intervals. One hundred larvae of both *H. virescens* and the backcross were reared individually in 30-ml cups containing 10 ml of wheat germ diet. Larval-stage duration tests were performed when the larvae were about 12 h old. Each instar was marked with a different-colored powder to aid in determining molting, and the cups were checked daily for larval development or pupation. Additional tests were performed with unmarked larvae to determine total larval period and survival, pupal period and weight, moth emergence, egg production, and moth longevity. Temperatures for these tests were 25°, 29.5°, and 34° C, and each treatment included 200 individuals of both *H. virescens* and the backcross. In the first tests, percentage of hatch for the backcross eggs was lower than for the *H. virescens* eggs at 16° C only. Duration of the egg stage was very similar for both at the four highest temperatures but was 1.5 days longer for the backcross at 16° C. Duration of the larval instars was progressively shorter at the higher temperatures for *H. virescens* and the backcross, and both required more instars before pupation at temperatures above 25° C. Survival rate was lower and stage duration was shorter at 29.5° and 34° C for the unmarked larvae of *H. virescens* and the backcross. Pupal weights of both were also lower at these temperatures, and percentage of emergence for the backcross was 100% at 29.5° C but only 8% at 34° C. Egg production of both *H. virescens* and backcross females was considerably lower at 29.5° and 34° C than at 25° C,

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[1]Research entomologist, Bioenvironmental Insect Control Laboratory, Science and Education Administration, U.S. Department of Agriculture, P.O. Box 225, Stoneville, Miss. 38776.

[2]Professor, Department of Agricultural Economics, Mississippi State University, Mississippi State 39762.

and adult longevity was generally lower at the two highest temperatures. In fact, longevity was about 50% lower for the *H. virescens* males at 34° C than at 25° C, a difference of only 9°. When compared with *H. virescens*, the backcross females had a decreased oviposition rate during the peak days after emergence, but the older backcross females outproduced *H. virescens* females during the last half of their life cycle. These data will enable present and future rearing facilities to use optimum temperatures in their programs and will also supply the basic information for developing population models on the *H. virescens* × backcross interaction in the native population. Index terms: *Heliothis subflexa* (Guenée), *Heliothis virescens* (F.), hybrid backcross progeny, insect rearing, temperature control.

INTRODUCTION

Laster et al. (1976) suggested that hybrid sterility might be used to suppress field populations of the tobacco budworm, *Heliothis virescens* (F.). Interspecific crosses between female *H. subflexa* (Guenée) and male *H. virescens* produce sterile-male and fertile-female hybrids (Laster 1972), and hybrid females backcrossed to *H. virescens* males produce sterile sons. Continued backcrossing of these females to *H. virescens* males did not restore the fertility in backcrossed males after 60 generations (Laster, personal communication). Since *H. virescens* is a major pest of many crops, particularly cotton, a pilot test was initiated in 1977 on St. Croix, U.S. Virgin Islands, to determine the feasibility of suppressing this pest by infusing the male sterile trait into the indigenous population through the release of backcross progeny. Consistent production of high-quality backcross moths is vital to the success of this test. Information is available on rearing of the tobacco budworm (Fye and McAda 1972, Butler and Hamilton 1976, Butler and Henneberry 1976, Raulston and Lingren 1972). We report herein on the comparative development of *H. virescens* and the backcross reared at five constant temperatures. The data will aid in rearing these insects and provide information for developing a population model on the *H. virescens* × backcross interaction in the native population.

METHODS

Backcross eggs were obtained from M. L. Laster, Mississippi Agricultural and Forestry Experiment Station, Stoneville, Miss., and *H. virescens* eggs from USDA's Bioenvironmental Insect Control Laboratory at Stoneville. The backcross had been reared by procedures similar to those described by Berger (1963) and the *H. virescens* by procedures described by Raulston and Lingren (1972).

Test insects were reared under a 14-h-light and 10-h-dark regime in environmental cabinets maintained at temperatures of 16°, 20°, 25°, 29.5°, and 34° C, with relative humidity at 60% for eggs, larvae, and pupae and at 80% to 85% for adults. Eggs (12-h-old) used in viability and incubation tests were collected from oviposition cloths, surface-sterilized with 0.2% NaOCl solution, and placed individually in 30-ml cups containing about 5-ml of 3% agar. Then the cups were placed in the cabinets. Each temperature treatment was replicated 5 times with 21 cups per replication. The cups were checked for newly hatched larvae at 8-h intervals. These larvae

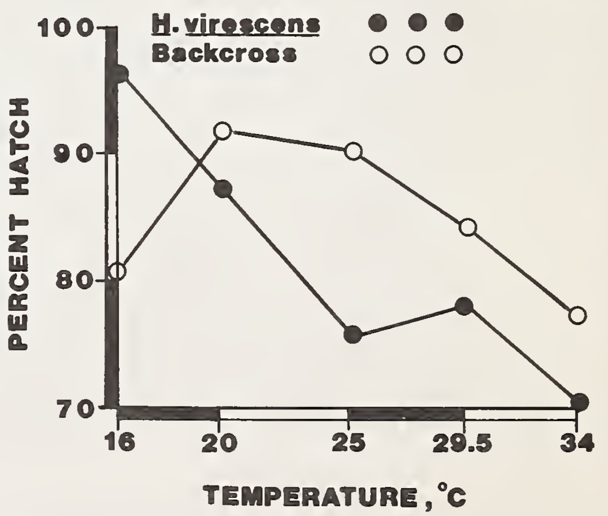

FIGURE 1.—Percentage of eggs of *H. virescens* and the backcross hatched at five temperatures.

were reared individually in 30-ml cups containing 10 ml of wheat germ diet (Brewer 1976) and sealed with wax-impregnated cardboard lids. Larval-stage duration tests were begun when the *H. virescens* and backcross larvae were about 12 h old. Each instar was marked with a different-colored, inert luminous powder to aid in determining molting (Fye and McAda 1972). One hundred larvae of both *H. virescens* and the backcross were reared at each of the five selected temperatures. The cups were checked daily for larval development or pupation. The sex of adults reared from larvae held at 25°, 29.5°, and 34° C was determined but was not determined for insects reared at the two lowest temperatures (16° and 20° C) because of low survival at these temperatures.

Additional tests were performed with unmarked larvae to determine total larval period and survival, pupal period and weights, moth emergence, egg production, and moth longevity. Temperatures for these tests were 25°, 29.5°, and 34° C, and each treatment included 200 individuals of both *H. virescens* and the backcross. Daily checks determined length of prepupal periods. Forty-eight hours after pupation the pupae were weighed, sexed, and returned to the temperature chambers for moth emergence. Following emergence, paired moths were placed in 0.5-l cardboard cartons and covered with cotton organdy lids. A cotton pad soaked daily with 5% sucrose-water solution was placed on the organdy cover as a food and water source. The organdy cover was changed daily to determine oviposition rate as total eggs laid per day per moth. Adult survival was also determined at this time. Dead females were dissected for determination of mating status by spermatophore count.

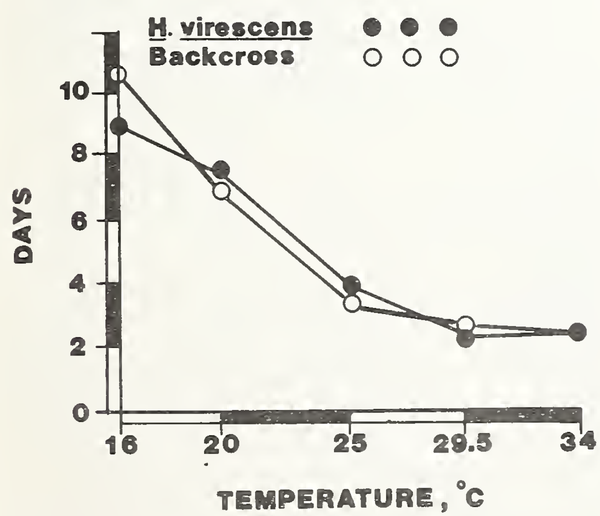

FIGURE 2.—Duration of viable eggs of *H. virescens* and the backcross at five temperatures.

RESULTS

Percentage of hatch for backcross eggs was less than that for *H. virescens* eggs at 16° C only (fig. 1). Duration of the egg stage was very similar for both the backcross and *H. virescens* at the four highest temperatures but differed by 1.5 d at 16° C (fig. 2). Table 1 shows the results of larval-stage duration tests at the two lowest temperatures. Duration of the larval instars was longer for the backcross than for *H. virescens* at 16° C, but it was very similar for both at 20°. The duration of each instar by sex for the three highest temperatures is presented in table 2.

Table 1.—Duration of larval instars of *H. virescens* and the backcross at the two lowest temperatures

Prepupal stage and insect	No. days at temperature of —	
	16° C	20° C
1st instar:		
H. virescens	6.5	4.5
Backcross	7.5	4.0
2d instar:		
H. virescens	5.1	4.0
Backcross	7.6	4.0
3d instar:		
H. virescens	5.2	4.1
Backcross	8.9	3.9
4th instar:		
H. virescens	6.8	4.3
Backcross	10.1	4.4
5th instar:[1]		
H. virescens	17.0	11.2
Backcross	([2])	12.0
Total:		
H. virescens	40.6	28.1
Backcross	....	28.3

[1]Duration of 6th stage combined with 5th stage. Average number of larval instars occurring at each temperature is given in table 3.

[2]Duration of 5th and 6th stages not recorded because of environmental cabinet malfunction before larvae pupated.

Table 2.—Duration of larval instars of *H. virescens* and the backcross by sex at the three highest temperatures

Prepupal stage and insect	No. days at temperature of —					
	25° C		29.5° C		34° C	
	♂	♀	♂	♀	♂	♀
1st instar:						
H. virescens ...	30	3.1	2.0	2.1	2.0	2.0
Backcross	2.8	3.0	2.2	2.3	2.0	2.1
2d instar:						
H. virescens ...	2.1	2.1	1.2	1.3	1.0	1.2
Backcross	2.2	2.1	1.6	1.8	1.7	1.6
3d instar:						
H. virescens ...	2.1	2.1	1.6	1.4	1.1	1.1
Backcross	2.3	2.2	1.6	1.3	1.2	1.4
4th instar:						
H. virescens ...	2.6	2.8	1.5	1.7	1.4	1.5
Backcross	2.8	2.7	1.9	2.1	2.2	1.6
5th instar:[1]						
H. virescens ...	7.1	6.5	4.5	4.1	4.3	4.6
Backcross	7.6	7.6	5.3	5.4	5.3	6.1
Total:[2]						
H. virescens	16.8 (43)	16.6 (32)	10.8 (31)	10.6 (38)	9.7 (22)	10.4 (26)
Backcross ..	17.6 (28)	17.6 (45)	12.5 (31)	12.9 (39)	12.3 (6)	12.9 (25)

[1]Duration of 6th stage combined with 5th stage. Average number of larval instars occurring at each temperature is given in table 3.

[2]Numbers in parentheses are actual numbers of larvae completing development to pupal stage and thus are used in calculations.

Table 3.—Number of larval instars required by *H. virescens* and the backcross before pupation when reared at five temperatures[1]

Temperature (°C)	Average No. instars	
	H. virescens	Backcross
16.0	5.4	....
20.0	5.4	5.3
25.0	5.1	5.1
29.5	5.5	5.8
34.0	5.5	5.6

[1]Averages based only on larvae completing development to pupal stage, except for the backcross at 16° C where test was terminated before larval pupation because of environmental cabinet malfunction.

Table 4.—Survival rate and duration of larval stage of unmarked *H. virescens* and the backcross by sex at three temperatures

Temperature and insect	Percentage of survival[1]	No. days duration	
		♂	♀
25° C:			
H. virescens	88	17.6	17.4
Backcross	88	17.9	17.9
29.5° C:			
H. virescens	80	11.6	11.4
Backcross	70	10.9	10.8
34° C:			
H. virescens	66	10.8	10.9
Backcross	65	11.4	11.4

[1]Percentage of larvae completing development to pupal stage.

Table 5.—Pupal weight and duration by sex and moth emergence rate of unmarked *H. virescens* and the backcross at three temperatures

Temperature and insect	Pupal weight (mg)[1]		No. days duration		Percentage of moth emergence
	♂	♀	♂	♀	
25° C:					
H. virescens	322.8 (89)	310.6 (86)	17.1	16.2	99
Backcross	320.8 (103)	317.2 (104)	18.1	16.3	99
29.5° C:					
H. virescens	302.4 (68)	297.2 (71)	11.5	10.6	100
Backcross	292.0 (59)	297.0 (55)	9.9	9.0	100
34° C:					
H. virescens	254.1 (61)	256.9 (65)	9.8	8.8	96
Backcross	292.9 (77)	292.3 (83)	11.0	9.9	88

[1]Numbers in parentheses indicate numbers of insects observed.

Duration was shortest at the highest temperatures for *H. virescens* and the backcross, with the greatest difference at 34° C. Also, *H. virescens* required 2.5 d less than did the backcross at this temperature. The average numbers of larval instars required before pupation for the backcross and *H. virescens* when reared at the five temperatures are given in table 3. Both insect groups tended to require more instars at temperatures above 25° C (the midrange temperature). Therefore, larvae held at near optimum conditions go through about five instars, while more extreme temperatures initiate an extra instar.

There were sharp decreases in the survival rate and duration of the unmarked larvae of *H. virescens* and the backcross at 29.5° and 34° C (table 4). Pupal weights were also lower at these temperatures, and percentage of emergence for the backcross was 100% at 29.5 ° C but only 88% at 34° C (table 5).

Egg production of both the backcross and *H. virescens* females was considerably lower at 29.5° and 34° C than at 25° C, and adult longevity was generally lower at the two highest temperatures (table 6). This was most apparent in the *H. virescens* males, where longevity was about 50% lower at 34° C than at 25° C, a difference of only 9°. When compared with *H. virescens*, the backcross females had a decreased oviposition rate during the peak days (5, 6, 7, and 8), but the older backcross females actually outproduced the *H. virescens* females during the last half of their life cycle (fig. 3).

Table 6.—Egg production and longevity of adults of unmarked *H. virescens* and the backcross at three temperatures[1]

Temperature and insect	No. eggs per ♀[2]	No. days longevity	
		♀	♂
25° C:			
H. virescens	959.6 (16)	15.7	19.0 (13)
Backcross	682.9 (16)	14.7	([3])
29.5° C:			
H. virescens	497.5 (17)	17.0	13.9 (18)
Backcross	523.4 (18)	16.1	([3])
34° C:			
H. virescens	100.3 (6)	11.6	9.5 (19)
Backcross	43.2 (6)	11.0	([3])

[1]Data compiled only from containers with a mated female.
[2]Numbers in parentheses indicate numbers of insects evaluated.
[3]Longevity of backcross males was not recorded.

REGRESSION ANALYSIS

Regression equations were calculated for converting the developmental data to mathematical expressions for use in simulation models. In these analyses of duration of each stage in life cycle, dependent variable Y represents the number of days required, and independent variable X expresses the temperature in degrees Fahrenheit. Y' represents the percentage of development per day of each stage. Utilizing regression equations to estimate Y or Y' is equivalent to making the prediction for $E(Y)$ or $E(Y')$ for a given X (temperature).

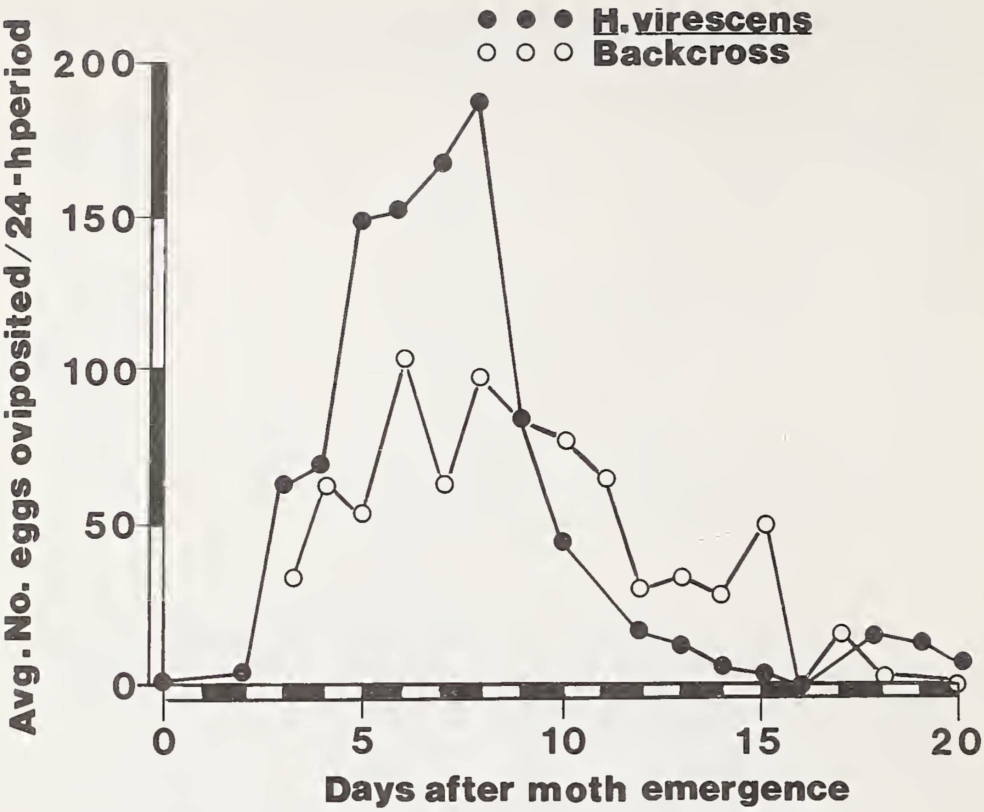

FIGURE 3.—Ovipositional cycles of unmarked *H. virescens* and the backcross at 25° C.

Expected-value operator E is a linear operator; therefore, the expectation of a function of random variables to be the function of their expectations does not hold for nonlinear functions (Kane 1968). Thus,

$$Y = 100/Y',$$

and

$$E(Y) = E(100/Y') = 100E(1/Y'),$$

but is not equal to $100/E(Y')$.

If the regression equation for percentage of development per day were applied to estimate the number of days required for each stage in the life cycle, it would be equivalent to utilizing $100/E(Y')$ to make the prediction for $E(100/Y')$. $E(100/Y')$ is not equal to $100/E(Y')$; therefore, to make the prediction in this way would sacrifice soundness in the statistical theoretical basis. Moreover, to maintain the soundness of theoretical basis in making the estimation, two kinds of regression equations are fitted, one for the duration of each stage and the other for the percentage of development per day in each stage of life cycle. These equations, with accompanying statistics, are given in tables A-1—A-4 of the appendix. The statistical models to be fitted are:

$$Y = \beta_0 + \beta_1 X + e$$

and

$$Y' = \beta_{0'} + \beta_1 X + e'.$$

This procedure may be applicable to model development for other insect species as well.

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APPENDIX.—REGRESSION ANALYSES

Table A-1.—Regression analysis of duration of each stage in life cycle, in days, for the backcross[1]

Stage	Sex	d.f.[2]	Regression equation	Temperature range (° F)	R^2	F value	T value for β_1	$\overline{X}$	$\sum_{i=1}^{n}(X_i-\overline{X})^2$	$\hat{\sigma}_{y \cdot x}$
Egg		219	Y=21.324-0.2153X	60.8-93.2	0.8484	1225.6	35.0	76.6538	30704.1	1.0776859
Larval	Male	64	Y=51.2428-0.43735X	77.0-93.2	.4658	55.8	7.5	82.5227	1857.96	2.52365083
	Female	107	Y=42.0538-0.317248X	77.0-93.2	.349	57.4	7.6	83.6138	4351.93	2.76309392
Pupal	Male	61	Y=43.9634-0.363998X	77.0-93.2	.3933	39.5	6.3	82.1429	1614.21	2.32545066
	Female	103	Y=35.221-0.269564X	77.0-93.2	.3817	63.6	8.0	83.6343	4301.52	2.21736681
Adult	Female	58	Y=32.8784-0.234568X	77.0-93.2	.0931	6.0	2.4	85.1	2624.4	4.92387451
Egg laying		52	Y=35953-0.297314X	77.0-93.2	.1847	11.8	3.4	84.8	2357.10	4.20625078

[1]Formula for calculating confidence interval for individual $\hat{Y}$ is

$$\hat{Y}-t\,\hat{\sigma}_{y \cdot x}\sqrt{1+\frac{1}{n}+\frac{(X_j-\overline{X})^2}{\sum_{i=1}^{n}(X_i-\overline{X})^2}} < Y < \hat{Y}+t\,\hat{\sigma}_{y \cdot x}\sqrt{1+\frac{1}{n}+\frac{(X_j-\overline{X})^2}{\sum_{i=1}^{n}(X_i-\overline{X})^2}}$$

[2]d.f., degrees of freedom.

Table A-2.—Regression analysis of each stage in life cycle, in percentage of development per day, for the backcross[1]

Stage	Sex	d.f.[2]	Regression equation	Temperature range (° F)	R^2	F value	T value for β_1	$\bar{X}$	$\sum_{i=1}^{n}(X_i-\bar{X})^2$	$\hat{\sigma}_{y \cdot x}$
Egg		219	$Y'=-60.1085+1.15364X$	60.8-93.2	0.9349	3147.2	56.1	76.6538	30704.1	3.60332791
Larval	Male	64	$Y'=8.35438+0.184381X$	77.0-93.2	.6186	103.8	10.2	82.5227	1857.96	0.78005723
	Female	107	$Y'=4.24569+1.30964X$	77.0-93.2	.429	80.4	9.0	83.6138	4351.93	0.96356417
Pupal	Male	61	$Y'=-6.31493+0.16769X$	77.0-93.2	.2218	17.4	4.2	82.1429	1614.21	1.61559882
	Female	103	$Y'=7.23358+0.185232X$	77.0-93.2	.4729	92.4	9.6	83.6343	4301.52	1.26388662
Adult	Female	58	$Y'=3.19841+1.141735X$	77.0-93.2	.0831	5.3	2.3	85.1	2624.4	3.16677424
Egg laying		52	$Y'=26.6049+0.451535X$	77.0-93.2	.1683	10.5	3.2	84.8	2357.1	6.75950635

[1]Formula for calculating confidence interval for individual $\hat{Y}'$ is

$$\hat{Y}'-t\,\hat{\sigma}_{y \cdot x}\sqrt{1+\frac{1}{n}+\frac{(X_j-\bar{X})^2}{\sum_{i=1}^{n}(X_i-\bar{X})^2}} < Y' < \hat{Y}'+t\,\hat{\sigma}_{y \cdot x}\sqrt{1+\frac{1}{n}+\frac{(X_j-\bar{X})^2}{\sum_{i=1}^{n}(X_i-\bar{X})^2}}$$

[2]d.f., degrees of freedom.

Table A-3.—Regression analysis of duration of each stage in life cycle, in days, for *H. virescens*[1]

Stage	Sex	d.f.[2]	Regression equation	Temperature range (° F)	R^2	*F* value	*T* value for β_1	$\bar{X}$	$\sum_{i=1}^{n}(X_i-\bar{X})^2$	$\hat{\sigma}_{y \cdot x}$
Egg		427	Y=25.1064–0.260485X	60.8–93.2	0.82	1949.8	44.2	76.6203	55270.1	1.38686023
Larval	Male	67	Y=46.3747–0.389613X	68.0–93.2	.4811	62.1	7.9	85.7913	2621.39	2.53084989
	Female	83	Y=42.8496–0.354821X	77.0–93.2	.5669	108.6	10.4	85.7671	3045.85	1.87872579
Pupal	Male	63	Y=42.012–0.347958X	77.0–93.2	.5586	79.7	8.9	85.7231	2139.9	1.80259496
	Female	103	Y=34.0189–0.263942X	77.0–93.2	.4035	55.5	7.4	85.6786	2989.94	1.93788459
Adult	Male	118	Y=67.6573–0.629815X	77.0–93.2	.2724	44.2	6.6	85.235	5246.61	6.86292411
	Female	56	Y=35.1509–0.250162X	77.0–93.2	.082	5.0	2.2	85.10	2493.18	5.58516887
Egg laying		46	Y=25.9828–0.167439X	77.0–93.2	.057	2.8	1.7	84.2562	1868.52	4.34106946

[1]Formula for calculating confidence interval for individual Y is given in footnote 1, table A-1.

[2]d.f., degrees of freedom.

Table A-4.—Regression analysis of each stage in life cycle, in percentage of development per day, for *H. virescens*[1]

Stage	Sex	d.f.[2]	Regression equation	Temperature range (° F)	R^2	F value	T value for β_1	$\bar{X}$	$\sum_{i=1}^{n}(X_i-\bar{X})^2$	$\hat{\sigma}_{y \cdot x}$
Egg		427	$Y'=-63.7476+1.19128X$	60.8–93.2	0.9515	8379.0	91.5	76.6203	55270.1	3.0595112
Larval	Male	67	$Y'=-8.01517+0.188739X$	68.0–93.2	.4425	53.2	7.3	85.7913	2621.39	1.32502625
	Female	83	$Y'=-8.7276+0.199942X$	77.0–93.2	.5319	94.3	9.7	85.7671	3045.85	1.13629402
Pupal	Male	63	$Y'=9.09373+0.205886X$	77.0–93.2	.534	72.2	8.5	85.7231	2139.9	1.1210225
	Female	82	$Y'=-6.59577+0.183636X$	77.0–93.2	.4024	55.2	7.4	85.6786	2989.94	1.35133471
Adult	Male	118	$Y'=-45.2763+0.654544X$	77.0–93.2	.2575	40.9	6.4	85.235	5246.61	7.41170186
	Female	56	$Y'=-4.44331+0.152729X$	77.0–93.2	.0696	4.2	2.0	85.1	2493.18	3.72684115
Egg laying		46	$Y'=-2.39686+0.141943X$	77.0–93.2	.0613	3.0	1.7	84.2562	1868.52	3.5415318

[1]Formula for calculating confidence interval for individual Y' is given in footnote 1, table A-2.

[2]d.f., degrees of freedom.